DEMONSTRATION OF THE CHEMOTROPISM OF POLLEN TUBES IN VITRO IN FOUR PLANT SPECIES[1]

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The phenomenon of chemotropism has been reported for pollen tubes (Beck and Joly, 1941; Brink, 1924; Lidforss, 1899; Miyoshi, 1894a; Molisch, 1893; and Tsao, 1949) for the spores of certain fungi (Graves, 1916; Miyoshi, 1894b) and for the organs of higher plants such as roots (Newcombe and Rhodes, 1904). Although numerous species of higher vascular plants have been studied, only about 10 species produced pollen which exhibited chemotropic characteristics toward some part of the pistil of the same species. Examples of this phenomenon were first reported at the end of the 19th century (Lidforss, 1899; Molisch, 1893). The literature up to 1924 has been reviewed by Brink. Tsao conducted an extensive study of the chemotropism of pollen tubes and reported her results in 1949 with a review of the literature up to that time. The chemical or chemicals responsible for the tropic response have never been isolated.

This present study was initiated as a survey of a small number of species of plants, most of which had never been studied previously, for the possibility of the occurrence of the chemotropic characteristic of the pollen tubes toward plant parts of the same species.

MATERIALS AND METHODS

In most of the research reported in the literature, a method in vitro was used to demonstrate chemotropism of pollen tubes. This usually consisted of placing a small segment of some part of the pistil or other floral part on a semisolid medium and then placing the pollen around this tissue.

The method of Tsao (1949) was used in this study with only minor modifications. Pollen was collected from plants grown in the greenhouse. Where possible, flowers just at anthesis or a day or so past anthesis were used in these studies. However, no attempt was made to use pollen of exactly the same age in the tests. Flowers were brought intact into the laboratory and the pistil, other floral organs, or plant parts were cut into small segments. The size of these segments varied, but for segments of the ovulary, for the petals and sepals, pieces about 3–5 mm square were used. The style provided small cylinders about 3 mm in length. Ovules, when used in these studies, were excised intact from the ovulary. The method consisted of placing about five segments from each floral organ at approximately equally spaced intervals on a semisolid medium in petri dishes. Pollen from the same species was then placed around the floral tissue segment at a distance of about 1 mm with a dissecting needle, under a binocular microscope. Thus, each test or treatment consisted of at least five duplicates in any one experiment and each experiment was repeated at least once. Positive chemotropism in a given species was arbitrarily considered as the growth of about 90 percent or more of the pollen tubes from grains placed 1 mm from the test segment toward that segment.

The medium consisted of 1 percent agar-agar, 10 percent sucrose, and 100 ppm Difco Yeast extract. The mixture was autoclaved for 15 minutes at 15 pounds pressure. Before the medium had cooled completely it was poured into

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petri dishes to a depth of about 4 mm. The plant materials were placed on the medium after it had cooled and the dishes were examined under low power at intervals beginning one hour after the pollen was placed on the medium. For species whose pollen did not germinate well, the dishes were examined the following day and then discarded. For species whose pollen germinated readily on the medium employed here, the results could usually be read in from two to four hours. No attempt was made to adjust the medium in order to obtain good growth of the pollen of all the species studied.

RESULTS

Ten species of plants, representing ten genera and seven families were studied. The results are shown in table 1. Positive chemotropism was demonstrated by pollen of one species of *Aloe*, one species of *Clivia*, one species of *Gasteria*, and one species of *Haworthia*, to segments of one or more floral organs of the same species

TABLE 1

The results of the study of the effect of one or more floral parts on the direction of growth of pollen of the same species in vitro

Plant	Family	Positive chemotropism
Aloe confusa	Liliaceae	+[1]
Antirrhinum majus	Scrophulariaceae	−[2]
Clivia nobilis	Amaryllidaceae	+
Crinum sp.	Amaryllidaceae	−
Epiphyllum sp.	Cactaceae	−
Gasteria verrucosa	Liliaceae	+
Haworthia planifolia	Liliaceae	+
Impatiens sultani	Balsaminaceae	−
Kalanchoe sp.	Crassulaceae	−
Nicotiana sp.	Solanaceae	−

[1]90% or more of the pollen tubes grew toward the floral part embedded in the medium.

[2]Growth of the pollen tubes was at random.

TABLE 2

Plant species in which pollen tube chemotropism was demonstrated

Plant	Tissues toward which pollen tubes of the same species grew
Aloe confusa	Stigma, style, petal, sepal
Clivia nobilis	Stigma, style, ovulary wall, ovule, leaf, petal, sepal
Gasteria verrucosa	Stigma, ovulary
Haworthia planifolia	Stigma, style, ovulary

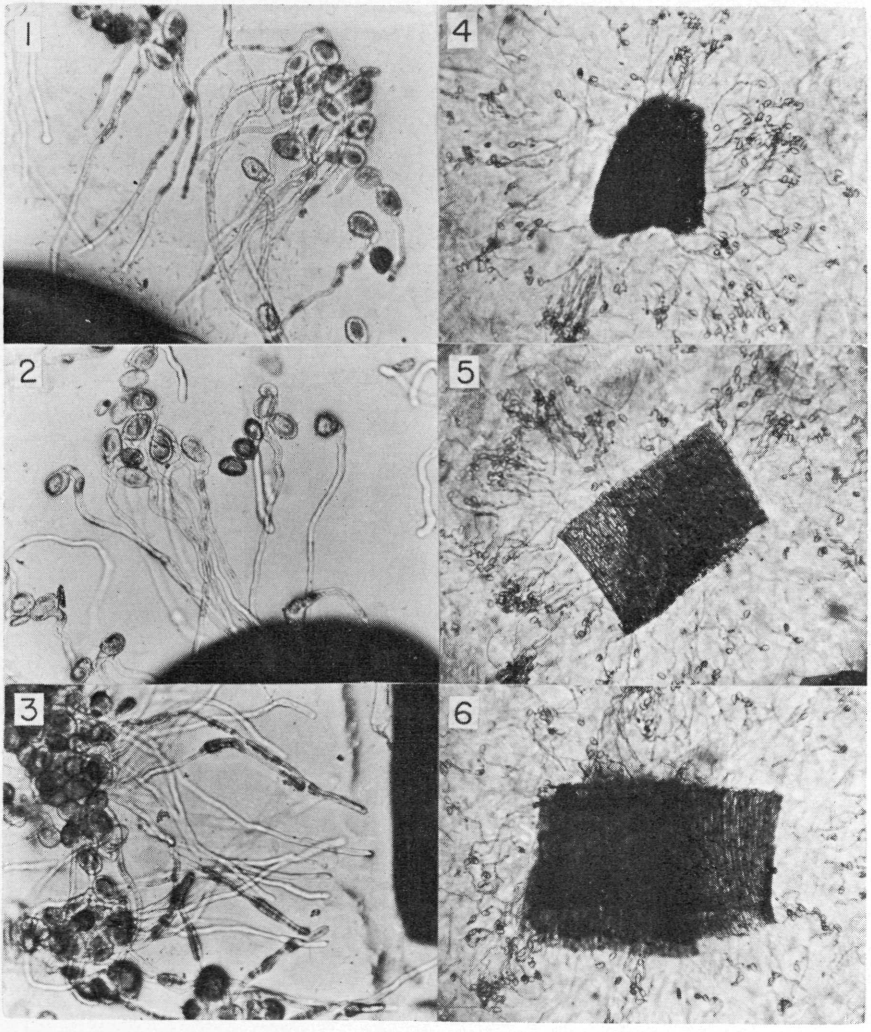
respectively. The pollen of six other species grew at random when placed around segments of floral organs of the same species.

In addition to parts of the ovulary such as the stigma, style, ovulary wall, and ovule, other floral parts such as petals and sepals, as well as leaf tissue for certain species, illicited the chemotropic response to pollen of the same species. These results are given in table 2.

Photomicrographs were made of the chemotropic response of the *Clivia nobilis* pollen to parts of the flower and to leaf segments (fig. 1–6).

Additional studies were carried out on the pollen of *Clivia nobilis* to learn more of the nature of the chemotropic factor.

With this species it was possible to confirm the results of Tsao (1949) that the chemotropic factor is heat stable and readily diffusable into agar. Even after heating segments of the ovulary in water at 212° F for 15 minutes, it was possible to obtain the tropic response to this tissue equivalent to the unheated controls. When ovules (6–12) were placed on agar cubes (about 4 mm^3 in volume) and allowed to stand for 5–8 hours, it was possible to obtain the chemotropic response of pollen of *Clivia nobilis* to these cubes *after* the ovules had been removed (fig. 7). Again the response was approximately equivalent to controls using excised intact ovules on agar plates as previously described. The pollen was placed approxi-



FIGURES 1–6. The demonstration of the growth of pollen tubes of *Clivia nobilis* toward vegetative and floral parts of the same species. FIGURES 1–3. Growth of the pollen tubes toward a segment of the style; toward an entire, excised ovule; and toward a segment of a leaf, respectively. FIGURES 4–6. Growth of the pollen tubes toward the stigma, toward a segment of the sepal, and toward a segment of the petal, respectively.

mately 1 mm from the agar cubes as in the other experiments with tissue segments. In order to test the possibility that pollen tubes of *Clivia nobilis* would grow toward any object embedded in the agar, experiments similar to those described were carried out with the tissue segments replaced by clean glass beads. Growth of the tubes of *Clivia nobilis* pollen grew completely at random around the glass beads.

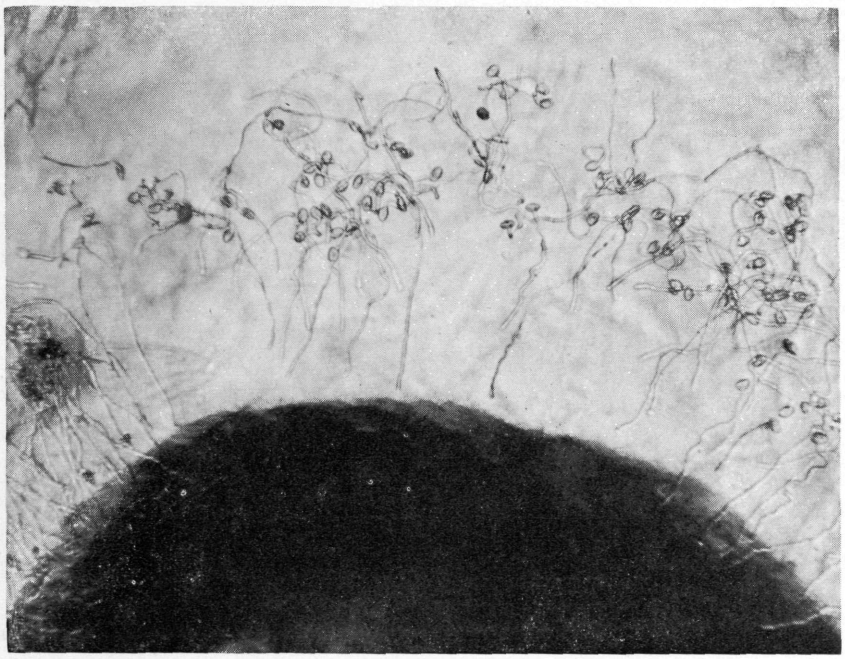


FIGURE 7. The demonstration of the growth of pollen tubes of *Clivia nobilis* toward an agar cube containing a diffusate from ovules of the same species.

DISCUSSION OF RESULTS

The data presented here in general corroborate the results of Tsao (1949). However, it was not possible to demonstrate chemotropism in the unidentified variety of *Antirrhinum majus* studied here. The active factor for *Clivia nobilis* may be chemically similar to the unknown chemical reported by Tsao (1949) for *Lilium superbum* since both are heat stable, water soluble, and diffusable into agar.

Tsao (1949) reported that the petals, sepals and leaves of *Antirrhinum majus* did not attract the pollen tubes of the same species. In the present study it was found that the sepal, petal, and leaf segments of *Clivia nobilis* did attract the pollen tubes of the same species. Thus, it appears that at least in this species the active factor is distributed throughout the above ground parts of the plant. It was also possible to demonstrate chemotropism of the pollen tubes of *Aloe confusa* to segments of the petal and sepal in addition to the tropic response to the stigma and to segments of the style (table 2).

SUMMARY

1. Of ten species of plants investigated, it was possible to demonstrate the chemotropism of the pollen tubes of four species to tissue segments of the same species. These four species are *Aloe confusa*, *Clivia nobilis*, *Gasteria verrucosa*, and *Haworthia planifolia*.

2. The pollen of these four species exhibited the chemotropic response to various segments of the pistils of the respective species, and in addition the pollen of *Aloe confusa* and *Clivia nobilis* grew toward petal and sepal segments. The pollen of *Clivia nobilis* also demonstrated the chemotropic response to leaf tissue segments.

3. The active factor bringing about the chemotropic growth of the pollen tubes of *Clivia nobilis* was found to be heat stable, water soluble, and diffusable into agar.

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